EXHIBIT A

REVIEW ARTICLE

MECHANISMS OF DISEASE

Mechanisms of Thrombus Formation

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HEMOSTASIS IS THE PROCESS THAT MAINTAINS THE INTEGRITY OF A closed, high-pressure circulatory system after vascular damage. Vessel-wall injury and the extravasation of blood from the circulation rapidly initiate events in the vessel wall and in blood that seal the breach. Circulating platelets are recruited to the site of injury, where they become a major component of the developing thrombus; blood coagulation, initiated by tissue factor, culminates in the generation of thrombin and fibrin. These events occur concomitantly (Fig. 1A; also see Video, available with the full text of this article at www.nejm.org), and under normal conditions, regulatory mechanisms contain thrombus formation temporally and spatially.

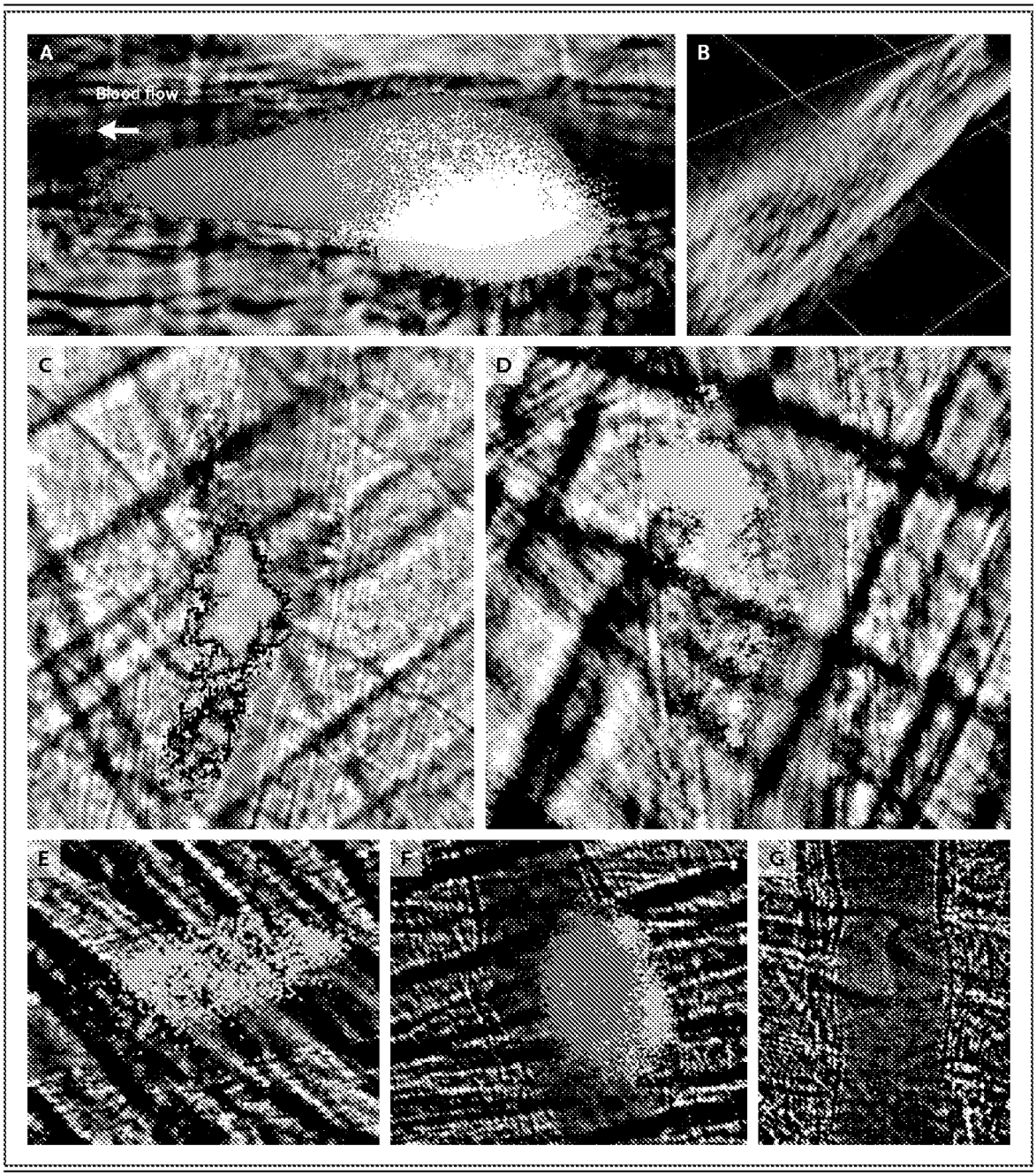
When pathologic processes overwhelm the regulatory mechanisms of hemostasis, excessive quantities of thrombin form, initiating thrombosis (Fig. 1B; and Video, Chap. 2). Thrombosis is a critical event in the arterial diseases associated with myocardial infarction and stroke, and venous thromboembolic disorders account for considerable morbidity and mortality. Moreover, venous thrombosis is the second leading cause of death in patients with cancer. Our understanding of the molecular and cellular basis of thrombus formation has advanced greatly through the use of novel techniques for studying mouse models of thrombosis. In this article, we review recent advances in knowledge about thrombus formation. We also offer new hypotheses and some speculations about thrombus formation and the prevention and treatment of thrombosis.

Figure 1 (facing page). **Thrombus Formation In Vivo.**

The developing thrombus in a living mouse after vessel-wall injury (Panel A) is characterized by the deposition of platelets (red), tissue factor (green), and fibrin (blue). Platelet thrombus formation and fibrin deposition occur concomitantly. Platelets and tissue factor appear yellow; tissue factor and fibrin, turquoise; platelets and fibrin, magenta; and platelets, fibrin, and tissue factor, white. A three-dimensional, confocal optical reconstruction of a thrombus in the lumen of an arteriole (Panel B) shows the platelet thrombus (red and yellow) being formed in the vessel wall, which is lined with the endothelium (labeled green with antibodies to platelet-endothelial cell-adhesion molecule [PECAM-1]). Platelets are labeled red using antibodies to CD41; platelets stained with both CD41 and PECAM-1 appear yellow. Calcium is mobilized during platelet activation. Panel C shows platelets loaded with a calcium-sensitive dye during thrombus formation; resting platelets appear green, and activated platelets appear yellow. Labeled microparticles bearing tissue factor (Panel D, green) infused into a recipient mouse accumulate in the developing thrombus. In Panel E, expression of protein disulfide isomerase (PDI, green) is shown during thrombus formation. Panel F shows fibrin (green) and platelets (red), which appear rapidly after vessel-wall injury and form a thrombus; yellow indicates colocalization of fibrin and platelets. In Panel G, inhibition of PDI blocks platelet accumulation and the generation of fibrin, and neither is observed. A video showing the process of thrombus formation in live mice is available with the full text of this article at www.nejm.org.

FORMATION OF A PLATELET THROMBUS

The vessel wall, with its inner lining of endothelium, is crucial to the maintenance of a patent vasculature. The endothelium contains three thromboregulators — nitric oxide,[1,2] prostacyclin,[3] and the ectonucleotidase CD39[4] — which together provide a defense against thrombus formation. Collagen in the subendothelial matrix and tissue factor facilitate the maintenance of a closed circulatory system. When the vessel wall is breached or the endothelium is disrupted, collagen and tissue factor become exposed to the flowing blood, thereby initiating formation of a thrombus (Fig. 2). Exposed collagen triggers the accumulation and activation of platelets, whereas exposed tissue factor initiates the generation of thrombin, which not only converts fibrinogen to fibrin but also activates platelets.



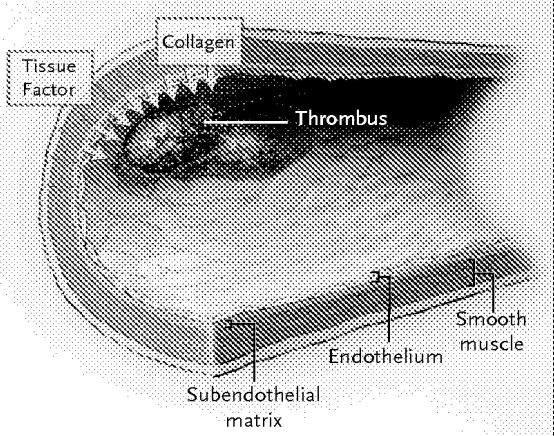


Figure 2. **Response to Vascular Injury.**

Collagen and tissue factor associated with the vessel wall provide a hemostatic barrier to maintain the high-pressure circulatory system. Collagen (yellow arrows), located in the subendothelial matrix beneath the endothelium, is not exposed to flowing blood under normal conditions. Tissue factor (blue arrows), located in the medial (smooth muscle) and adventitial layers of the vessel wall, comes in contact with flowing blood when the vessel is disrupted or punctured. Both collagen and thrombin initiate thrombus formation. Collagen is a first line of defense, and tissue factor a second line of defense.

TWO INDEPENDENT PATHWAYS TO PLATELET ACTIVATION

The idea that two distinct pathways acting in parallel or separately can activate platelets derives from recent studies of thrombus formation in genetically altered mice.[5,6] In one of these pathways, exposure of subendothelial collagen initiates platelet activation; in the other, thrombin — generated by tissue factor derived from the vessel wall or present in flowing blood — is the initiator (Fig. 3). Depending on the injury or the disease, one pathway or the other may predominate, but the consequences of platelet activation triggered by these pathways are the same.

The interactions of platelet glycoprotein VI (see Glossary) with the collagen of the exposed vessel wall and of platelet glycoprotein Ib-V-IX with collagen-bound von Willebrand factor result in adhesion of platelets to the site of injury (Fig. 3). The relative importance of platelet glycoproteins VI and Ib-V-IX in the initial tethering of platelets depends on the shear rate at the vessel wall.[7] However, the interaction of collagen with glycoprotein VI is required, as is glycoprotein Ib, a component of the glycoprotein Ib-V-IX complex.[5,8,9] In addition to its role in the adherence of platelets to collagen, glycoprotein VI is the major agonist for initial platelet activation and granule release. The platelet integrin $\alpha_2\beta_1$ plays a supportive but not essential role in the interaction between platelets and collagen.[10,11] Platelet activation in this collagen-initiated pathway is independent of thrombin.

Tissue factor triggers a second pathway that initiates platelet activation (Fig. 3). Platelet activation initiated by this pathway does not require disruption of the endothelium and is independent of von Willebrand factor[12] and glycoprotein VI.[5] Experiments in mice have shown that only some of the adherent platelets become activated (Fig. 1C; and Video, Chap. 3) and that the activation of these platelets is independent of von Willebrand factor.[12] Tissue factor forms a complex with factor VIIa, the enzymatically active form of factor VII, and this tissue factor–factor VIIa complex activates factor IX, thereby initiating a proteolytic cascade that generates thrombin. Thrombin cleaves protease-activated receptor 4 (Par4 [Par1 in humans]) on the platelet surface, thereby activating platelets[13] and causing them to release adenosine diphosphate (ADP), serotonin, and thromboxane A_2. In turn, these agonists activate other platelets, and in so doing, amplify the signals for thrombus formation. This second pathway does not explain how platelets are recruited to a site of vessel injury where collagen is not exposed to flowing blood. Perhaps the initiating insult induces endothelial cells lining the affected vessel to display adhesive molecules that tether platelets to the injured endothelium.

Specific experimental conditions can cause thrombus formation exclusively through the collagen or tissue factor pathway.[5,6] The exact contribution of the two pathways to platelet activation is unknown, however, and their participation may vary with the underlying disease. Because of the redundancy of mechanisms that activate platelets, inhibitors of such targets as glycoprotein VI in the collagen pathway or blood-clotting enzymes in the tissue factor pathway may not provide protection against platelet activation in all disorders.

PROPAGATION OF THE PLATELET THROMBUS

A developing thrombus recruits unstimulated platelets,[12] and within the thrombus activation

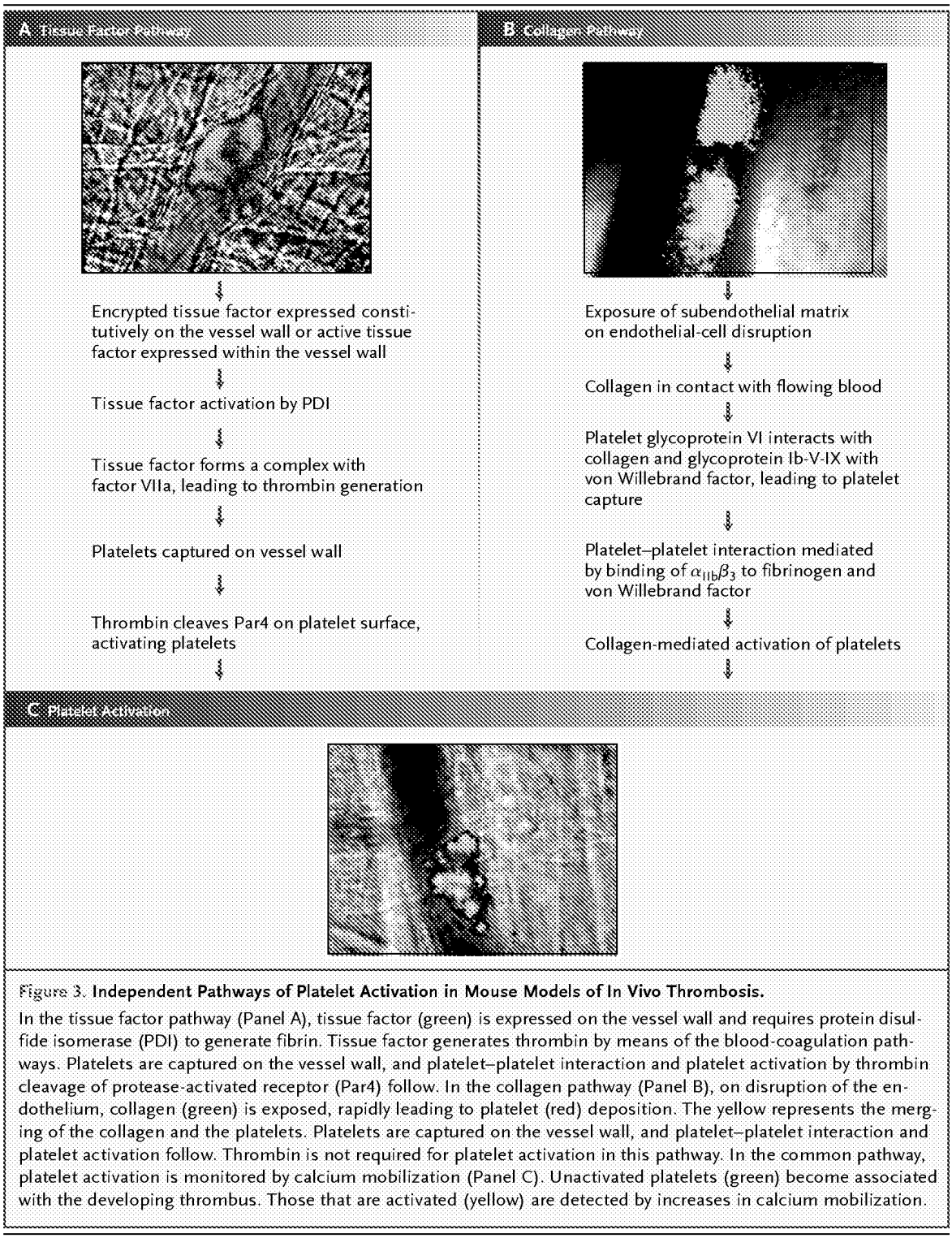


Figure 3. Independent Pathways of Platelet Activation in Mouse Models of In Vivo Thrombosis.

In the tissue factor pathway (Panel A), tissue factor (green) is expressed on the vessel wall and requires protein disulfide isomerase (PDI) to generate fibrin. Tissue factor generates thrombin by means of the blood-coagulation pathways. Platelets are captured on the vessel wall, and platelet–platelet interaction and platelet activation by thrombin cleavage of protease-activated receptor (Par4) follow. In the collagen pathway (Panel B), on disruption of the endothelium, collagen (green) is exposed, rapidly leading to platelet (red) deposition. The yellow represents the merging of the collagen and the platelets. Platelets are captured on the vessel wall, and platelet–platelet interaction and platelet activation follow. Thrombin is not required for platelet activation in this pathway. In the common pathway, platelet activation is monitored by calcium mobilization (Panel C). Unactivated platelets (green) become associated with the developing thrombus. Those that are activated (yellow) are detected by increases in calcium mobilization.

occurs only in a subgroup of the recruited platelets. Others remain loosely associated with the thrombus but do not undergo activation and may ultimately disengage from the thrombus (Fig. 1C).[12] In short, thrombus formation is a dynamic process in which some platelets adhere to and others separate from the developing thrombus, and in which shear, flow, turbulence, and the number of platelets in the circulation greatly influence the architecture of the clot.

Glossary

Adenosine diphosphate (ADP): An agonist of platelet activation.

$\alpha_2\beta_1$: A platelet integrin that plays a role in collagen binding.

$\alpha_{IIb}\beta_3$: An integrin that serves as the fibrinogen receptor on platelets.

β_3 integrin: A subunit of integrins.

CD36: A leukocyte surface protein.

CD40 ligand: A platelet receptor for CD40 that triggers an inflammatory response.

CD55: A leukocyte surface protein associated with the cell membrane by means of a glycerolinositol anchor. The anchor is defective in paroxysmal nocturnal hemoglobinuria.

CD59: A leukocyte surface protein associated with the cell membrane by means of a glycerolinositol anchor. The anchor is defective in paroxysmal nocturnal hemoglobinuria.

Ephrin-Eph: Receptor kinases and ligands on platelet surfaces.

Glycoprotein Ib-V-IX: A cluster of adhesive receptors on platelets. Von Willebrand factor binds to this complex.

Glycoprotein VI: A collagen receptor on platelets.

Growth-arrest–specific gene 6: A vitamin K–dependent membrane protein involved in cell signaling.

Protease-activated receptor 1 (Par1): A thrombin receptor on platelets; equivalent to Par4 on mouse platelets; activation initiates cell-signaling pathways.

P-selectin: An adhesion molecule on activated platelets and endothelial cells that binds to PSGL-1, its receptor on leukocytes.

P-selectin glycoprotein ligand 1 (PSGL-1): An adhesion molecule on leukocytes that binds to P-selectin.

P2Y1: An ADP receptor on platelets.

P2Y12: An ADP receptor on platelets.

Signaling lymphocyte activation molecule: An adhesion molecule found on platelets.

Tissue factor: A cytokine receptor analogue on the surface of cells that initiates blood coagulation and is engaged in cell-signaling events.

Tissue factor pathway inhibitor: A protein that, when bound to factor Xa, blocks the activity of the tissue factor–factor VIIa complex.

Von Willebrand factor: A plasma protein that is the carrier for factor VIII and that is critical for the adhesion of platelets to the vessel wall.

The platelet integrin $\alpha_{IIb}\beta_3$, when activated, mediates recruitment of platelets to the thrombus as well as platelet–platelet interactions. Activation of $\alpha_{IIb}\beta_3$ requires an enzyme (protein disulfide isomerase) that catalyzes cleavage or formation of disulfide bonds between cysteine residues.[14-16] Activation of platelets bound to the wall of the injured vessel causes a conformational transition in $\alpha_{IIb}\beta_3$ that increases the affinity of the integrin for its ligands, fibrinogen and von Willebrand factor.[17] At low shear rates, fibrinogen is the predominant ligand, whereas von Willebrand factor plays an important role at higher shear rates.[7,18] However, neither von Willebrand factor nor fibrinogen is absolutely required for platelet accumulation.[19] In addition, von Willebrand factor is a ligand for glycoprotein Ib, but results in a mouse thrombosis model involving denudation of the endothelium suggest an as yet unidentified alternative ligand for glycoprotein Ib.[9] During platelet activation, late signaling events enhance platelet–platelet affinity. Growth-arrest–specific gene 6,[20] CD40 ligand,[21] ephrin-Eph,[22] and signaling lymphocyte activation molecule[23] participate in the platelet–platelet synapse to create a protected environment in the interstices of the clot that stabilizes the thrombus.[24]

Platelet activation releases the contents of platelet alpha granules and dense granules, each of which carries a cargo of components that are critical for thrombus formation. Proteins are packaged in various subpopulations of alpha granules,[25] whereas ADP and calcium ions are packaged in the dense granules. The release of ADP stimulates platelet activation through two ADP receptors, P2Y1 and P2Y12. The role of these receptors in platelet function and the pharmacology of drugs directed against these receptors has recently been reviewed.[26]

BLOOD COAGULATION

TISSUE FACTOR

A membrane protein, tissue factor is present on cells in numerous anatomical compartments and has multiple functions. In addition to initiating blood coagulation, tissue factor mediates intracellular signaling events that are important for angiogenesis,[27] tumor progression,[28] metastasis,[29] and maintenance of the yolk-sac vasculature.[30] Tissue factor is constitutively expressed on fibroblasts and pericytes in the adventitia and medial smooth-muscle cells of the vessel wall. It is also constitutively expressed on many nonvascular cells, and its expression on monocytes and endothelial cells can be induced by chemical stimuli.[31,32] The idea that there may be functionally significant amounts of tissue factor on granulocytes and platelets remains controversial.[33] The endothelium was thought to act as a barrier separating factor VIIa in flowing blood from cellular sources of tissue factor in order to prevent the initiation of coagulation in the absence of injury.[34] However, tissue factor is also present in circulating blood, and this bloodborne tissue factor may participate in physiologic and pathologic processes.[35]

Tissue factor is associated with some micro-

particles in the circulating blood.[35,36] These vesicular structures, which are less than 1000 nm in diameter, display proteins of the blood cells from which they were derived (e.g., leukocytes, platelets, endothelial cells, smooth-muscle cells, and monocytes).[36,37] During thrombus formation, platelets accumulate at the vessel wall, become activated, and express P-selectin.[38] This adhesion molecule binds to microparticles that display the P-selectin counterreceptor, termed P-selectin glycoprotein ligand 1 (PSGL-1), allowing the thrombus to capture microparticles that display tissue factor derived from monocytes (Fig. 1D; and Video, Chap. 4).[36] Fibrin propagation within the thrombus is dominated by bloodborne tissue factor when vessel-wall injury is limited to endothelial-cell activation.[39]

What prevents tissue factor on microparticles from initiating blood coagulation? Tissue factor can exist in a latent (or "encrypted") form that lacks coagulant activity or in an active form that initiates blood coagulation.[40,41] The molecular basis of encryption is uncertain, but dimerization,[42] lipid reorganization,[43] and cellular secretion of tissue factor–rich granules[44] are among the proposed mechanisms. One of the two disulfide bonds in tissue factor may be a labile allosteric disulfide bond[45] that can undergo cleavage or formation, with effects on the structure and function of the protein.[46,47] Oxidation of free thiols in encrypted tissue factor to form a disulfide bond yields a conformation that allows the tissue factor–factor VIIa complex to bind to and activate factor X.[45,48] How can these altered disulfide bonds explain the transformation of bloodborne tissue factor from the encrypted to the active form in response to vessel-wall injury? Activated endothelial cells and platelets at the site of injury release protein disulfide isomerase, which catalyzes the formation and breakage of disulfide bonds between cysteine residues within proteins (Fig. 1E; and Video, Chap. 5).[49] This enzyme is required for fibrin generation and platelet thrombus formation (Fig. 1F and 1G; and Video, Chap. 6). Perhaps it acts by promoting the formation of a functionally critical disulfide bond in tissue factor.

THROMBIN AND FIBRIN

Tissue factor is the sole initiator of thrombin generation and fibrin formation. The contact pathway of blood coagulation,[50,51] a powerful tool for in vitro studies of the coagulation cascade, is not required for initiation of hemostasis in vivo.[52] A complete deficiency of factor XII, high-molecular-weight kininogen, or prekallekrein is associated with major defects in the initiation of the contact pathway of coagulation, as manifested by a markedly prolonged partial-thromboplastin time. Nevertheless, patients with any one of these deficiencies do not have a hemorrhagic disorder. The importance of factor XII in thrombosis remains controversial, but in mice, a deficiency of factor XII or factor XI attenuates the development of thrombi.[53-55] Furthermore, inhibition or deficiency of factor XII protects mice from ischemic brain injury without causing hemorrhage.[56] In humans, factor XI deficiency may be associated with a hemorrhagic phenotype. Factor XI may also participate in thrombosis in humans, because a deficiency of this protein is associated with a reduced risk of ischemic stroke but not of myocardial infarction.[57]

A new iteration of the coagulation pathways is required to accommodate these findings and hypotheses (Fig. 4). We propose that the activation of encrypted tissue factor by protein disulfide isomerase initiates coagulation. On activation, platelets and endothelial cells secrete the isomerase,[49] which converts inactive tissue factor on cells or microparticles to its active form. In the case of direct tissue damage, tissue factor in the vessel wall or on cell surfaces may already exist in its active form, and the isomerase may not be required. This tissue factor pathway can be considered the fuse that ignites coagulation with a small amount of thrombin.[62] Other salient features of these coagulation pathways indicate that the sole initiator of thrombin generation is active tissue factor. Before thrombin is generated, the tissue factor pathway, proceeding through factor IX or factor X, is inefficient because factors VIII and V, the circulating pro-cofactors required in the tenase and prothrombinase complexes, are not yet available in their most active cofactor form. This inefficient mechanism generates a small amount of thrombin. Once formed, thrombin converts factors VIII and V to their cofactor forms, factor VIIIa and factor Va, respectively. The tenase and prothrombinase complexes now proceed efficiently to generate a large burst of thrombin. The tissue factor pathway is down-regulated, or inhibited, by the action of tissue factor pathway inhibitor, but thrombin generation proceeds without replenishing active tissue factor.[63]

The question of what promotes continued thrombin generation in the absence of continued production of the active tissue factor–factor VIIa complex is unresolved. In vitro studies that can mimic thrombus formation in flowing blood by resupplying coagulation proteins in the absence of additional tissue factor and in the presence of factor XIIa inhibitors suggest that the prothrombinase formed when tissue factor–factor VIIa ignites coagulation can sustain continued thrombin generation. This newly formed thrombin feeds back to activate factors VIII and V, which form factors VIIIa and Va, triggering the greatly amplified formation of additional thrombin through the pathway mediated by the fully active tenase and prothrombinase complexes. Alternatively, factor XI, which is activated by thrombin,[64] creates a reservoir of initiator activity after the tissue factor pathway is terminated.[62] However, the ability of thrombin to activate factor XI in plasma has been questioned.[65]

What are the membrane surfaces on which the tenase and prothrombinase complexes assemble? It has been thought that the membrane surface that is critical for thrombin generation is presented by the activated platelet. However, fibrin generation in the Par4-null mouse, whose platelets cannot be activated by thrombin, is normal,[66] suggesting the importance of other membrane surfaces in vivo. Factor XII and factor XI are less important for hemostasis than for thrombosis[52]; nevertheless, this framework includes an important, albeit not critical, role of factor XI in hemostasis. Three questions remain unanswered: What activates factor XII during thrombosis, and why is activation of this zymogen not important during hemostasis? What enzyme is responsible for the constitutive circulation of factor VIIa? What are the cellular surfaces on which the tenase and prothrombinase complexes assemble if activated platelets are not required?

Figure 4 (facing page). **Pathways of Blood Coagulation during Hemostasis and Thrombosis.**

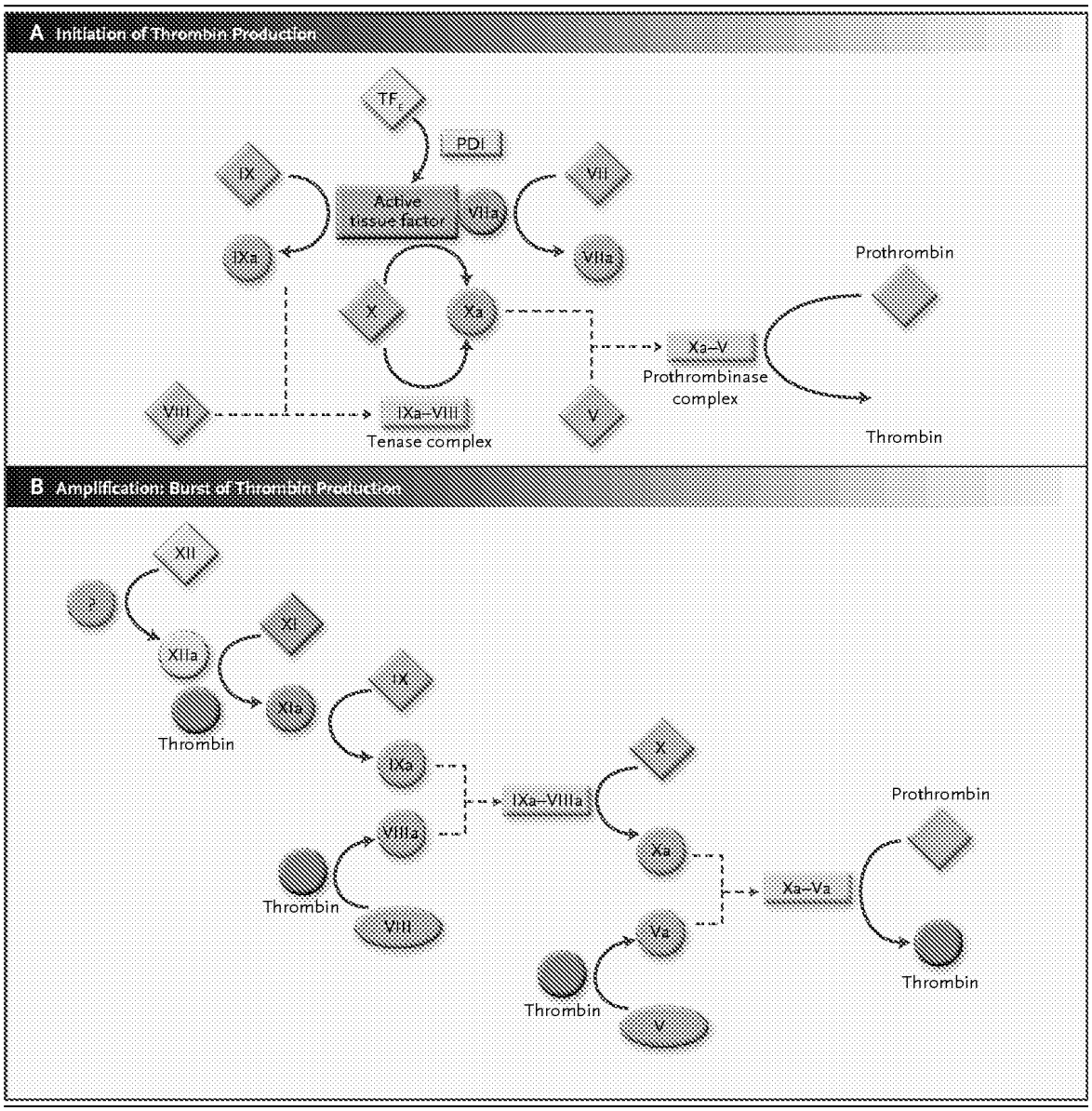
Coagulation can be divided into the initiation phase (Panel A) and the amplification phase (Panel B). During initiation, the tissue factor–factor VIIa complex serves as a fuse to trigger blood coagulation by generating small amounts of thrombin. Although the mechanism is not known, in this pathway the protein disulfide isomerase (PDI) pathway is required for thrombin generation. Tissue factor forms a complex with circulating factor VIIa.[34] This complex, which plays a major role in coagulation, has three substrates: factor VII, factor IX,[58] and factor X. Factor IXa binds to factor VIII.[59] This complex inefficiently activates factor X to form factor Xa. Factor Xa, generated by the tissue factor–factor VIIa complex or the factor IXa–factor VIII complex, binds factor V on membrane surfaces. This complex converts prothrombin to thrombin. The rate of thrombin generation with factor V is less than 1% of the rate of thrombin generation in the presence of thrombin-activated factor Va.[60,61] During amplification, the thrombin generated activates factors VIII and V, leading to a burst of thrombin-generating potential. Alternatively, or in addition, thrombin may activate factor XI. During hemostasis, the tissue factor pathway that is the fuse for initiation of coagulation is inactivated. The tenase complex and prothrombinase complex efficiently generate the large thrombin burst. In some mechanisms of thrombosis, tissue factor may require activation by protein disulfide isomerase, whereas in other mechanisms, active tissue factor may be available as a consequence of a related disease process. TF_E denotes encrypted tissue factor.

TISSUE FACTOR AND MICROPARTICLES IN THROMBOTIC DISORDERS

Acute inflammation and infection, sepsis, and endotoxemia can induce a hypercoagulable state. When regulatory mechanisms are overwhelmed, acute disseminated intravascular coagulation ensues, with consumption of blood coagulation proteins and platelets and, hence, bleeding. In the chronic form of disseminated intravascular coagulation, thrombosis rather than hemorrhage is predominant. Thrombosis and inflammation are related and mutually reinforcing processes, involving inflammatory mediators (e.g., endotoxin, tumor necrosis factor α, and CD40 ligand), tissue factor expression on monocytes and the activated endothelium,[67] and circulating tissue factor–bearing microparticles.[37] A primary cause of thrombosis in disseminated intravascular coagulation is disruption of endogenous anticoagulant pathways.

HEMOSTATIC MICROPARTICLES VERSUS PATHOLOGIC MICROPARTICLES

There is no detectable tissue factor activity in normal blood,[68] yet tissue factor–bearing microparticles circulate in healthy persons. Perhaps tissue factor–bearing microparticles contain inactive tissue factor, which may become activated only when the particles are recruited to the site of vascular injury (Fig. 5A). Pathologic microparticles may bear active tissue factor, which may confer a predisposition to thromboembolic events. Mi-



croparticles bearing tissue factor derived from tumor cells or inflammatory cells can cause thrombotic events,[37] and circulating microparticles bearing active tissue factor may be a biomarker for an increased thrombotic risk (Fig. 5B). The presence of high levels of such microparticles warrant consideration as the predisposing cause of thrombosis in a variety of disorders.

CANCER-ASSOCIATED THROMBOSIS

The molecular and cellular basis of the association of thrombosis with cancer is uncertain. Proposed causes for the increased risk of thrombosis in cancer include activation of blood coagulation by tissue factor in tumors,[70] a factor X–activating cysteine protease,[71] mucinous glycoproteins,[72] *MET* oncogene activation,[73] and circulating tumor-derived, tissue factor–bearing microparticles.[69,74,75] A pilot study supports the hypothesis that elevated numbers of tumor-derived, tissue factor–bearing microparticles in plasma contribute to cancer-associated thrombosis (unpublished data). Although epithelial-derived tumors do not express PSGL-1, other mucinous glycoproteins are components of adenocarcinomas[72] and are probably surface components of tumor-derived microparticles that bind to P-selectin.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Thrombosis of the hepatic and portal circulation is a feature of paroxysmal nocturnal hemoglobinuria. The abundance of procoagulant, leuko-

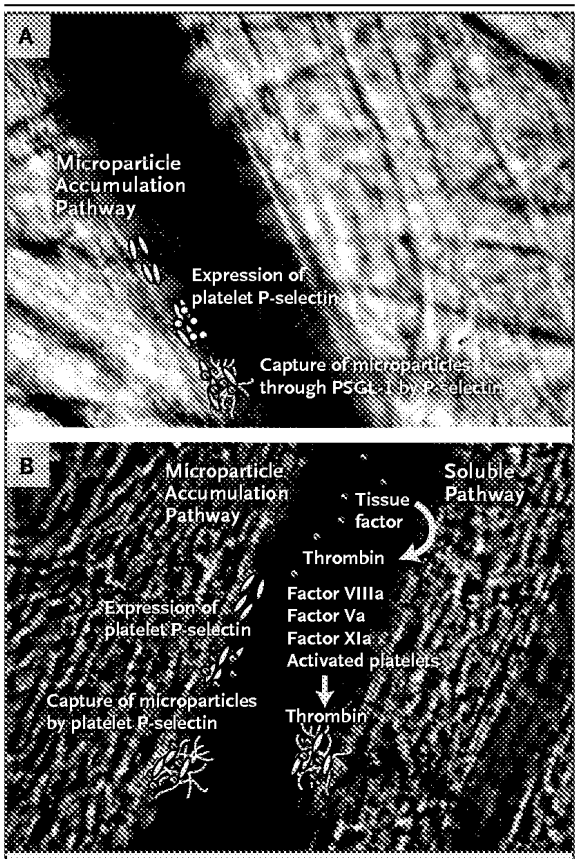


Figure 5. **Hemostatic versus Pathologic Microparticles.**
In this emerging model, hemostatic microparticles (Panel A, white) are constitutive, are present at a low concentration, and may express inactive tissue factor. The capture of these microparticles in the developing thrombus, mediated by platelets (yellow) through P-selectin and microparticle P-selectin glycoprotein ligand 1 (PSGL-1), leads to their accumulation at the injury site and results in activation of microparticle tissue factor (red).[36] In contrast, pathologic microparticles (Panel B, red) express active tissue factor[69] and are present at a high concentration in the circulation. These microparticles accumulate, perhaps by binding to activated platelets (yellow); expressing P-selectin, which captures microparticles through an as yet unidentified mucinous glycoprotein that is an analogue of the mucinous glycoprotein PSGL-1; or by binding to activated endothelium. In either case, tissue factor can lead to thrombin generation. Thrombin generation leads to platelet thrombus formation and fibrin (blue) generation. However, as pathologic microparticles bear active tissue factor, they do not require the activation step that may regulate formation of thrombin by action of hemostatic microparticles.

cyte-derived microparticles in the circulation of patients with this disorder suggests that the microparticles derive from complement-injured CD55-deficient and CD59-deficient monocytes and macrophages expressing tissue factor.[76,77]

ATHEROTHROMBOSIS

Atherosclerosis would be a chronic disorder associated with reduced blood flow to target organs as a result of stenotic lesions, without serious morbidity or mortality, if it were not for the thrombotic event, the major pathogenic process in acute coronary artery disease.[78] Chronic atherosclerotic lesions of the coronary arterial wall are diffuse and associated with the formation of both obstructive and nonobstructive plaque. The currently favored hypothesis is that rupture of the fibrous cap of the plaque initiates thrombus formation by exposing blood to collagen in the extracellular matrix, to previously sequestered tissue factor associated with lipid-laden macrophages, or both.[79,80] Tissue factor, a component of atheroma,[81] is important in coronary thrombosis; in an animal model of coronary injury, tissue factor pathway inhibitor reduced the size of the thrombus.[82] The source of tissue factor at sites of plaque rupture has been inferred from anatomical analyses of pathological specimens after fatal myocardial infarction. However, the diffusion rates of proteins involved in blood coagulation are too slow for tissue factor to migrate from the ruptured plaque into the growing thrombus.[83] A proposed alternative mechanism is that tissue factor in the thrombus derives from tissue factor–bearing microparticles that bind to activated platelets at the site of plaque rupture, perhaps by binding of platelet P-selectin to microparticle PSGL-1.[35,36,84] Plaque rupture may be associated with activation of tissue factor from its encrypted form on microparticles. Oxidized lipids, such as choline glycerophospholipids, are implicated in platelet activation through CD36,[85] and thrombus formation is blocked when extracellular protein disulfide isomerase is inhibited, perhaps preventing the activation of critical functions in platelet receptors and tissue factor.[49]

NEW STRATEGIES FOR ANTITHROMBOTIC AGENTS

New pharmacologic agents, the most advanced of which are directed against factor Xa or thrombin,[86] have the potential to displace warfarin, heparin, and low-molecular-weight heparin for the treatment of and prophylaxis against thromboembolic disease. These new agents promise improved convenience, safety, and equal or improved efficacy. However, the targets of these

inhibitors are the same as those of heparin and warfarin, and they may compromise hemostasis, thereby causing hemorrhage while preventing thrombosis.

The ideal antithrombotic agent would inhibit thrombosis but spare hemostasis. Mechanisms of thrombosis differ among the various predisposing conditions. The development of optimal pharmacologic agents for the prevention of thrombosis associated with a particular disease should include consideration of specific mechanisms. Since injury to the vessel wall is the major hemostatic challenge, independent strategies for blocking pathologic thrombosis should focus on pathways that do not involve repair of breached vessels. Activated factor XII might be an example of a target for new inhibitors of thrombin generation: occlusive thrombi do not form in mice lacking factor XII,[53,56] and neither mice nor humans who are deficient in factor XII have a hemostatic defect. The evidence implicating microparticles that display tumor-derived or monocyte-derived tissue factor in the thrombotic complications of cancer or inflammation suggests that such particles could be suitable targets for thromboprophylaxis. In a mouse model of laser-induced vascular injury, inhibition of the reaction between P-selectin and the PSGL-1 receptor has been shown to block the accumulation of microparticles bearing monocyte-derived tissue factor in a developing thrombus.[36] Although not yet tested clinically, this observation suggests the possibility that pharmacologic inhibitors that disrupt the P-selectin–PSGL-1 interaction may have the potential to act as antithrombotic agents, especially in disorders that are associated with activation of endothelial cells but in which the integrity of the endothelium is preserved.[87-89] Inhibition of tissue factor or prevention of microparticle accumulation might provide prophylactic treatment against cancer-associated thrombosis.

Thrombosis remains a final pathway to disease and death in some of our most common diseases: myocardial infarction, stroke, and cancer. Although substantial progress has been made in understanding the biology of thrombus formation and the pathophysiology of thrombosis, all the pharmacologic agents available for prevention or treatment have been in use for decades or have been replaced with newer variants that offer a modest incremental improvement. The ideal drug for prophylaxis and treatment of thrombotic disease remains an agent that will inhibit thrombosis but not hemostasis. The translation of new knowledge from in vitro and in vivo studies in animal models to pharmaceutical development presents opportunities for substantial advances in the prevention of thrombotic diseases.

Dr. Bruce Furie and Dr. Barbara Furie report receiving annual licensing fees for patents on P-selectin. No other potential conflict of interest relevant to this article was reported.

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